Ricerca means Research

REPORT

Study Title:

Determination of Water Solubility of K32

Ricerca Study Number: 035239

Ricerca Document Number: 035239-1

Data Requirement:

EPA OPPTS Test Guidelines 830.7840
OECD TGL.105

Study Completed:
February 02, 2017

Author:
Ling-Jen Ferguson, Ph.D.

Testing Facility:
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7528 Auburn Road
Ricerca Biosciences, LLC
Concord OH 44077, USA

Study Sponsor:
Koch Agronomic Services, LLC
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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA section 10 (d) (1) (A), (B) or (C), and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provision regarding disclosure to multinational entities under FIFRA 10(g).

Company: Koch Agronomic Services, LLC
Sponsor/submitter: Eric A. Searcy
Title: Product Regulatory Manager

Eric A. Searcy 2/2/2017
Signature **Date**
Koch Agronomic Services, LLC

These data are the property of Koch Agronomic Services, LLC and, as such, are considered confidential for all purposes other than compliance with FIFRA Section 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute or in any other country.

COMPLIANCE STATEMENT

The study reported herein, "Determination of Water Solubility of K32," Ricerca Document Number 035239-1, was conducted and reported in compliance with the Good Laboratory Practice Regulations set forth in Title 40, Part 160 of the Code of Federal Regulations of the United States of America.

The regulatory standards required for this study are compatible with the OECD GLP Principles and the multilateral agreement of the Mutual Acceptance of Data (MAD) System allows the submission of the data & report of this study to be submitted to appropriate OECD member countries.

Ling-Jen Ferguson — Feb. 2, 2017

Ling-Jen Ferguson, Ph.D., Study Director — **Date**
Ricerca Biosciences, LLC

Eric A. Searcy — 2/2/2017

Eric Searcy, Sponsor Representative — **Date**
Koch Agronomic Services, LLC

Eric A. Searcy — 2/2/2017

Submitter — **Date**
Koch Agronomic Services, LLC

QUALITY ASSURANCE UNIT STATEMENT

The Ricerca Quality Assurance Unit has performed inspections on the study, "Determination of Water Solubility of K32" Ricerca Study Number 035239. The results of these inspections, including any findings or observations, were reported to the Study Director and Management for appropriate corrective actions on the dates listed below:

Phase of Study Inspected	Dates Inspected	Dates Reported to Study Director	Dates Reported to Management
Protocol	Sept 9, 2016	Sept 9, 2016	Sept 9, 2016
In-Study	Oct 26, 2016	Oct 26, 2016	Oct 26, 2016
Data and Report	Jan 27, 2017	Jan 27, 2017	Jan 27, 2017

C L O'Leary — February 2, 2017

Ann L. O'Leary, Ph.D.
Quality Assurance Auditor

Date

APPROVAL

Study Title: Determination of Water Solubility of K32

Document Number: 035239-1

Testing Facility: Ricerca Biosciences, LLC
7528 Auburn Road
Concord, OH 44077

Ling-Jen Ferguson — Feb. 2, 2017

Ling-Jen Ferguson, Ph.D., Study Director — **Date**
Ricerca Biosciences, LLC

Robert McClanahan — 2 Feb 2017

Robert McClanahan, Ph.D., Management — **Date**
Ricerca Biosciences, LLC

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SUMMARY

Determination of the solubility of K32 in purified water was carried out. The study was performed following EPA OPPTS Test Guidelines 830.7840 and OECD TGL.105. K32 is a multiple component substance. The preliminary test of K32 (9.9 mg) in purified water (1 L) showed there were undissolved parts of K32 in water, indicating the water solubility of K32 at ambient temperature was < 10 mg/L. As the water solubility of K32 could not be determined from the preliminary test, 50 mg/L of K32 in purified water (5x the guideline requirement of the lowest testing concentration (10 mg/L) for the preliminary test) in triplicate was prepared for the definitive shake flask method. ICP-AES analysis of the samples from the definitive test showed the phosphorus content to be 4.4 ± 0.5 µg/mL (n = 3), which is equivalent to 71 ± 9% (n = 3) of the phosphorus in K32 added to the water (6.2 µg/mL). The stability of K32 components at the test conditions is demonstrated though HPLC analysis of a K32 sample in acetonitrile-HPLC water (1:1) at Time 0 and after 96 hours at 30 °C. In conclusion, many components in K32 are soluble and stable in water, although not all components are water soluble.

STUDY INFORMATION

STUDY TITLE

Determination of Water Solubility of K32

RICERCA STUDY NUMBER

035239

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SCHEDULE OF EVENTS

Study Initiation Date:	September 30, 2016
Experimental Start Date:	October 11, 2016
Experimental Completion Date:	November 1, 2016
Study Completion Date:	February 02, 2017

RETENTION OF DATA

Upon completion of the study, the complete study file, including all original raw data, protocol, and final report, will be submitted to the Ricerca Biosciences, LLC archives. All non-study-specific raw data (e.g., instrument logs) are archived at Ricerca Biosciences, LLC.

CONDUCT OF THE STUDY

The study was conducted at the Ricerca Biosciences, LLC AgChem Product Development Department Laboratories according to the Ricerca Biosciences, LLC protocol "Determination of Water Solubility of K32" (Ricerca document number 035239-0), located in Appendix A.

Personnel involved with the study were:

Ling-Jen Ferguson	Scientist
Richard L. Shea	Associate Scientist I
Kenneth Furlong	Associate Scientist III

INTRODUCTION

PURPOSE AND OBJECTIVES

The purpose of this study was to determine the water solubility of K32.

MATERIALS AND METHODS

TEST SUBSTANCE

- **K32**

Composition:	Reaction products of NBPT with urea and formaldehyde
Batch/Lot Number:	55700-30-13
Analyzed Concentration:	NBPT 20.04 wt%; Reaction product mixtures ~80 wt%
% Phosphorus Content	12.4%
Manufactured by:	Ricerca Biosciences
Date of manufacture:	July 20, 2016
Appearance:	Off-white to pale yellow gel
Storage:	Refrigerated

K32 (Lot No. 55700-30-13) was synthesized by Ricerca (Study No. 034689). GLP characterization of K32 was performed under Ricerca Study No. 035469. A certificate of analysis (CoA) of K32 from Ricerca Study No. 035469 is included in Appendix B.

STUDY OVERVIEW

If no literature value or previous data is available, a preliminary test to estimate the solubility should be conducted. In the preliminary test, the water solubility of the material at ambient temperature is determined by visually inspecting for any undissolved parts of the material in water. If the sample remains undissolved, an increasing volume of water will be added.

Dilutions will be continued until it is documented that the estimated solubility is $<10^{-2}$ g/L (< 10 mg/L).

In the definitive study (shake flask method), the material is dissolved in water at a temperature (30 ˚C) somewhat above the test temperature (20 °C). When saturation is achieved, the mixture is cooled and kept at the test temperature to reach equilibrium. The mass concentration of the substance in the aqueous solution is determined. The quantity of the material necessary to saturate the desired volume of water is estimated from the preliminary test. About 5x the quantity of material determined in the preliminary test is weighed into three glass vessels. The closed vessels are then agitated at 30 °C using a shaking device. After 1 day, one of the vessels is removed and re-equilibrated for 24 hours at the test temperature (20 °C) with occasional agitation. The contents of the vessel are then centrifuged at the test temperature (20 °C), and the concentration of the material in the clear aqueous phase is determined. The other two flasks are treated similarly after initial equilibration at 30 °C for 2 and 3 days, respectively. The concentration results from at least the last two vessels should not differ by more than 15%.

REAGENTS

- Water: RICCA Chemical (Arlington, TX) ASTM Type I, ASTM Type II, Double Distilled, ACS Reagent Grade, Cat. No.9150-1, Lot#: 4608H62 Exp.: 2/2018 (referred as “purified water” in this report)
- Water: Fisher, HPLC Grade (referred as “HPLC water” in this report)
- Water: House Deionized Water (referred as “deionized water” in this report)
- Acetonitrile: Sigma Aldrich, HPLC Grade
- 1000 µg/mL NIST-traceable P std reference solution (CPI 4400-1000391)

EQUIPMENT

- Analytical Balance
- Centrifuge: Sorvall Legend X1R (the temperature set at 20 ˚C)
- Incubator/shaker maintained at 30 ˚C
- Environmental chamber maintained at 20 ˚C
- Perkin Elmer Optima 3000 Dual-View Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES)
- PerkinElmer HPLC 200 series
- Standard glass lab ware (flask, graduated cylinders, etc.)

PRELIMINARY TEST

The quantity of the test substance necessary to saturate the desired volume of water was estimated from the preliminary test. A preliminary test was conducted by mixing a known amount of K32 (9.9 mg) with 1 L of purified water at ambient temperature for 5 days with occasional shaking.

DEFINITIVE TEST (SHAKE FLASK METHOD)

Approximately 10-mg aliquots of K32 were weighed into three separate 250 mL glass bottles and ~200 mL each of purified water was added (i.e. 50 mg/L water). The actual K32 weights and water volume are shown in the table below. The samples were agitated at 30 ˚C (i.e. magnetic stirring in an incubator set at 30 ˚C).

Sample	K32 Weight (mg)	Purified Water (mL)
Water Solubility Day 1	10.3	206
Water Solubility Day 2	10.0	200
Water Solubility Day3	10.9	218

At time points of 24, 48, and 72 hours, one sample each was removed from incubation/stirring at 30˚C and then stored at 20 ˚C for 24 hours with occasionally shaking.

After equilibrating for 24 hours at 20 ˚C, an aliquot from each sample was placed in a glass centrifuge tube and centrifuged at 2400 rpm, 20 ˚C for 10 min. After centrifugation, an aliquot of the clear aqueous phase was placed in a glass vial for ICP-AES analysis to determine phosphorus content. pH values of each sample were measured using a pH indicator strip (colorpHast EMD cat. 9582, pH 4.0-7.0) and recorded.

STABILITY AT TEST CONDITIONS

K32 (10.6 mg) was weighed into a 125-mL glass bottle and 19.3 mL of acetonitrile-HPLC water (1:1, v:v) was added (i.e. 0.55 mg/mL). The sample was sonicated for 5 min and analyzed by HPLC-UV. The sample was then agitated at 30˚C (i.e. magnetic stirring in an incubator set at 30 ˚C) for 4 days (96 hours). After 96 hours, the sample was removed from incubation/stirring at 30 ˚C and then stored at 20 ˚C for 24 hours with occasionally shaking. After equilibrating for 24 hours at 20 ˚C, the sample was analyzed by HPLC-UV.

ANALYTICAL METHODOLOGY

ICP-AES Analysis

ICP-AES using an external standard methodology was employed to evaluate the phosphorus (P) contents in the test solutions.

Preparation of Samples

Samples were analyzed as received without further preparation.

Preparation of Calibration Standards

Calibration standards containing 0, 10, 20, and 30 µg/mL of P in deionized water were prepared by pipetting 0, 1.00, 2.00, and 3.00 mL of a 1000 µg/mL NIST-traceable P std reference solution (CPI 4400-1000391, lot 15C024, exp. 11/22/16) to four 100-mL volumetric flasks and house deionized water was added to volume.

Sample Analysis

Samples were analyzed against the calibration curves on the Perkin Elmer Optima 8300 Dual-View Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES). The samples and calibration standards were analyzed in "radial" mode, three replicates. Three independent wavelengths (213.617 nm, 214.914 nm, and 178.221 nm) were evaluated.

HPLC-UV System

HPLC:PerkinElmer HPLC 200 series

Column:	Restek Ultra C18 3 µm 4.6 x 150 mm
Column Temp:	Ambient
Flow Rate:	1 mL/min
Injection Volume:	20 µL (in acetonitrile-HPLC water (1:1))
UV Detection:	214 nm (1.0000 AUFS)
Run Time:	43 minutes
Mobile Phase A:	HPLC water
Mobile Phase B:	acetonitrile
Autosampler Flush:	methanol
Solvent Gradient:	

Time (minutes)	Flow rate (mL/minute)	% A	% B
0	1	87	13
8	1	87	13
30	1	30	70
31	1	87	13
43	1	87	13

RESULTS

DEFINITION AND UNITS

The solubility in water is specified by the saturation mass concentration of the substance in water, and is a function of temperature. Solubility is specified in units of mass per volume of solution, and is reported as miligrams per litre (mg/L), which is equivalent to µg/mL.

PRELIMINARY TEST

The quantity of the test substance (K32) necessary to saturate the desired volume of water for the definitive shake flask method is estimated from the preliminary test. In the preliminary test, the water solubility of a material at ambient temperature is determined by visually inspecting for any undissolved parts of the material in water. A preliminary test was conducted by placing a known mass of K32 (9.9 mg) in 1 L of purified water for at ambient temperature 5 days with occasional shaking. In the preliminary test, it was determined that K32 was not totally soluble in water at the concentration of 9.9 mg/L after 120 hours at ambient temperature. Therefore, the water solubility of K32 at ambient temperature is < 10 mg/L.

DEFINITIVE TEST (SHAKE FLASK METHOD)

The preliminary test indicated K32 was not totally soluble in water at the concentration of 9.9 mg/L. As the water solubility of K32 could not be determined from the preliminary test, 50 mg/L of K32 in purified water (5x the guideline requirement of the lowest testing concentration (10 mg/L) for the preliminary test) in triplicate was prepared for the definitive shake flask method. The three samples were agitated at 30 °C and removed from the 30 °C incubator after 24, 48, and 72 hours. Following equilibration at 20 °C for 24 hours, the samples were analyzed by ICP-AES and the pH values determined.

The ICP-AES was calibrated with P std reference solutions at three wavelengths (213.617 nm, 214.914 nm, and 178.221 nm) and the result is shown in Table 1. The detector calibration was found to be linear over the range 10 to 30 µg/mL of P in deionized water.

Table 1: ICP-AES Calibration Result

Wavelength (nm)	Correlation coefficient	LOQ (µg/mL)	10 µg/mL std
213.617	0.999999	0.4	9.972
214.914	0.999997	0.4	9.893
178.221	1.000000	0.5	9.936

Based on the evaluation, all three wavelengths were appropriate for analysis and the reported sample analysis results in Table 2 are the average values from all three wavelengths. The average phosphorus content in 24 hr, 48 hr, and 72 hr samples is 4.4 ± 0.5 µg/mL (n = 3), which is equivalent to 71 ± 9% (n = 3) of the phosphorus in K32 added to the water (6.2 µg/mL). The amount of phosphorus introduced is equivalent to 50 mg/L (or µg/mL) x 12.4% (the phosphorus content of K32 is 12.4%, see CoA in Appendix B).

Table 2: Phosphorus Content of Test Solutions

Solvent	Test Substance	Phosphorus content measured by ICP (µg/mL)	Phosphorus Introduced (µg/mL)*	% Phosphorus Soluble in Water
Water (Day 1)	K32	4.6	6.2	74
Water (Day 2)	K32	4.8	6.2	77
Water (Day 3)	K32	3.8	6.2	61
Average ± Standard Deviation		**4.4 ± 0.5**		**71 ± 9**

Day 1, Day 2 and Day 3 correspond to 24 hr, 48 hr and 72 hr samples, respectively.
**K32 introduced: 50 mg/L, so phosphorus introduced: 50 mg/L x 12.4% = 6.2 mg/L (or µg/mL)*

Table 3 shows the pH measurement of the three samples using pH indicator strips.

Table 3: pH of Test Solutions

Solvent	Test Substance	pH
Water (Day 1)	K32	4.7
Water (Day 2)	K32	4.7
Water (Day 3)	K32	4.7

Day 1, Day 2 and Day 3 correspond to 24 hr, 48 hr and 72 hr samples, respectively.
The pH was measured using pH indicator strips (EMD ColorpHast cat. 9582 pH 4.0-7.0).

STABILITY AT TEST CONDITIONS

K32 in acetonitrile-HPLC water (1:1, v:v) at the concentration of 0.55 mg/mL was prepared for stability assessment by HPLC-UV. The sample preparation and the HPLC method followed the procedure described in Ricerca Study No. 034689. The stability sample was analyzed by HPLC-UV before placed in a 30 °C incubator (Time 0). The sample was then agitated at 30 °C and removed from the 30 °C incubator after 96 hours. Following equilibration at 20 °C for 24 hours, the sample was analyzed by HPLC-UV. The HPLC chromatograms are shown in Figure 1 and the peak area % (total area normalization) is summarized in Table 4. The results demonstrate stability of K32 components at the test conditions.

Table 4: Stability of K32 at Test Conditions: Percentages of K32 Components at Time 0 and after 96 h at 30 °C

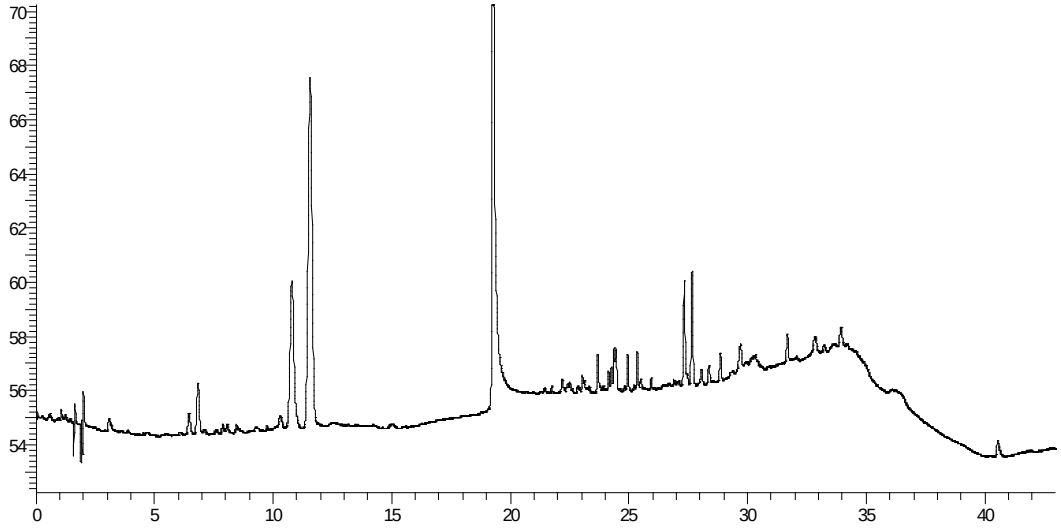
HPLC Retention Time (min)	HPLC Area %	
	Time 0	**30 °C, 96 h**
6.46	1.40	1.39
6.83	3.56	3.08
10.29	0.99	0.94
10.79	16.58	14.94
11.56	39.23	40.32
~22-33	38.24	39.35

CONCLUSION

Determination of the solubility of K32, a multiple component substance, in purified water was carried out. The preliminary test of K32 (9.9 mg) in purified water (1 L) showed there were undissolved parts of K32 in water, indicating the water solubility of K32 at ambient temperature was < 10 mg/L. As the water solubility of K32 could not be determined from the preliminary test, 50 mg/L of K32 in purified water (5x the guideline requirement of the lowest testing concentration (10 mg/L) for the preliminary test) in triplicate was prepared for the definitive shake flask method. ICP-AES analysis of the samples from the definitive test showed the phosphorus content to be 4.4 ± 0.5 µg/mL (n = 3), which is equivalent to 71 ± 9% (n = 3) of the phosphorus in K32 added to the water (6.2 µg/mL). The stability of K32 components at the test conditions is demonstrated though HPLC analysis of a K32 sample in acetonitrile-HPLC water (1:1) at Time 0 and after 96 hours at 30 °C. In conclusion, many components in K32 are soluble and stable in water, although not all components are water soluble.

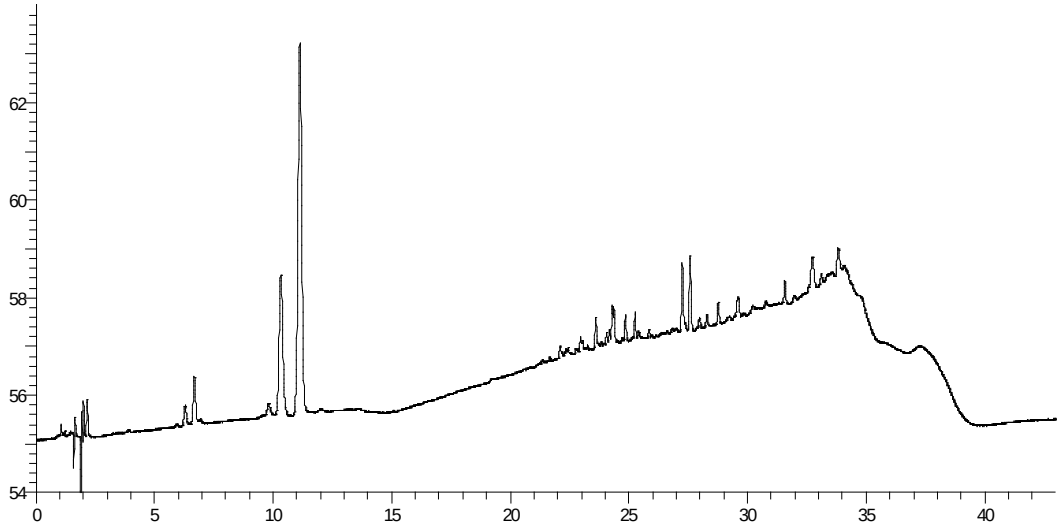
Figure 1: Stability of K32 at Test Conditions: HPLC Analysis of 0.55 mg/mL of K32 in ACN-HPLC Water (1:1) at Time 0 and after 96 h at 30 °C

HPLC run: Proj035239\10-27-16\01-uv-003
K32 0.55 mg/mL ACN-HPLC water (1:1) pre-test



The peak at ~19 min was also present in the blank run.

HPLC run: Proj035239\11-1-16\01-uv-003
K32 0.55 mg/mL ACN-HPLC water (1:1) 30C 96h



APPENDIX A

Protocol

PROTOCOL

Study Title:

Determination of Water Solubility of K32

Ricerca Study Number: 035239
Ricerca Document Number: 035239-0

Data Requirement:

EPA OPPTS Test Guidelines 830.7840
OECD TGL.105

Testing Facility:
AgChem Product Development
Ricerca Biosciences, LLC
Auburn Road
Concord OH 44077, USA

Study Sponsor:
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INTRODUCTION

K32 is a new product candidate developed by Koch Agronomic Services, LLC. K32 is a mixture of reaction products that incorporate N-(n-butyl) thiophosphoric triamide (NBPT), the active ingredient in AGROTAIN®, a nitrogen stabilizer product currently marketed by Koch Agronomic Services, LLC.

This study will develop data to help predict the test substance's mobility and ready access to humans and other living organisms by measurement of its water solubility.

PURPOSE

The purpose of this study is to determine the water solubility of K32. The study will be conducted to meet the registration requirement for US EPA, US EPA Guideline OPPTS 830.7840 Water Solubility: Column Elution Method; Shake Flask Method (Product Properties Test Guidelines) and OECD Guidelines for the Testing of Chemicals Test No. 105: Water Solubility (adopted 27 July1995).

STUDY INFORMATION

STUDY TITLE
Determination of Water Solubility of K32

RICERCA STUDY NUMBER
035239

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Regulatory Compliance

This study will be conducted in accordance with the U.S. EPA Good Laboratory Practice (GLP) Standards, 40 CFR 160.

Schedule of Events

Proposed Experimental Start Date: September 2016
Proposed Experimental Completion Date: October 2016

The actual starting and completion dates will be documented in the final report.

MATERIALS AND METHODS

Test Substance

Information concerning the test substance, K32, is provided as follows.

- **K32**

Composition:	Reaction products of NBPT with urea and formaldehyde
Batch/Lot Number:	55700-30-13
Analyzed Concentration:	Reaction product mixtures 80.3 wt%, NBPT 17.3 wt%, water 2.4 wt%
Manufactured by:	Ricerca Biosciences
Date of manufacture:	July 20, 2016
Appearance:	Off-white to pale yellow gel
Storage:	Refrigerated

Storage and Distribution

The Sponsor has supplied the test substance, which was stored at conditions specified above. All preparations will be made in a manner to preclude contamination or deterioration of the test substance. All solutions prepared from the test substance will be uniquely identified.

Upon completion of the study any portion of the test substance not utilized in the study will remain in storage at Ricerca Biosciences unless otherwise directed by the Sponsor.

Characterization of Test Substance

It is the responsibility of the Sponsor to characterize the test substance. The Sponsor will assume the responsibility of retention of a sample of the test material, as specified in 40 CFR 160.195.

Justification for Selection of Test System

The test procedures have been selected to comply with US EPA registration requirement: United States Environmental Protection Agency Product Properties Test Guidelines, 1996.

EXPERIMENTAL DESIGN

Overview

Water solubility is an important property, together with other physical and chemical properties, governing the tendency of a chemical to move and distribute between the various environmental compartments. In general, highly water-soluble chemicals are more likely to be transported and distributed by the hydrologic cycle than are relatively water-insoluble chemicals.

The solubility of a solid or a liquid chemical can be defined as the maximum amount of the chemical (the solute) in solution and at equilibrium with excess chemical in a solvent at specified ambient conditions.

Solubility is expressed as the weight of the solute per weight or volume of solvent (e.g., weight in milligrams, grams, or kilograms, and volume in milliliters or liters). Other concentration terms such as molarity (moles/L), molality (moles/1000 g), and percent solution (g/100 mL x 100) are also commonly used as solubility expressions.

The solubility at 20 °C (±0.5 °C) of the test substance in purified water will be determined and reported. The following detail is provided as a guideline for the conduct of the study. Good scientific judgment may be applied to optimize the experimental results. The Study Director will document all changes in or revisions of this approved protocol. To prevent plastic adsorption glass will be used when possible. The actual procedure will be summarized in the final report.

Preliminary Test

If no literature value or previous data is available, a preliminary test to estimate the solubility will be conducted.

To approximately 0.1 g of the sample (solid substances must be pulverized) in a stoppered 10-mL vessel, increasing volumes of water at room temperature are added according to the steps shown in the following table.

Solubility Data	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
Total Volume of Water Added (mL)	0.1	0.5	1	2	10	100	>100
Approximate Solubility (grams per liter)	>1000	1000 to 200	200 to 100	100 to 50	50 to 10	10 to 1	<1

After each addition of the indicated amount of solvent, the mixture is shaken vigorously for 10 minutes and is visually checked for any undissolved parts of the sample. If, after a total of 10 mL of water has been added (Step 5), the sample or parts of it remain undissolved, the contents of the measuring cylinder are transferred to a 100-mL measuring cylinder which is then filled up with water to 100 mL (Step 6) and shaken. At lower solubility the time required to dissolve a substance can be considerably longer (at least 24 hours should be allowed). The approximate solubility is given in the table under that volume of the added solvent in which complete dissolution of the sample occurs. If the substance is still apparently insoluble, more than 24 hours should be allowed (96 hours maximum), or further dilution should be undertaken. Dilutions will be continued until it is documented that the estimated solubility is $<10^{-2}$ g/L.

No single method is available to cover the whole range of solubility in water, from relatively soluble to very low soluble chemicals. As K32 contains 17.3 wt% NBPT and NBPT is water soluble, the "shake flask method" that applies to substances with higher solubility ($>10^{-2}$ g/L) will be performed to determine the water solubility of K32.

SHAKE FLASK METHOD

In this method, the compound is dissolved in water at a temperature (30˚C) somewhat above the test temperature (20 °C). When saturation is achieved, the mixture is cooled and kept at the test temperature, stirring as long as necessary to reach equilibrium. Subsequently, the mass concentration of the substance in the aqueous solution, which must not contain any undissolved particles, is determined by a suitable analytical method. The test can also be performed at the test temperature if it is assured, by appropriate sampling, that the saturation equilibrium is reached.

The quantity of material necessary to saturate the desired volume of water is estimated from the preliminary test. The volume of water required will depend on the analytical method and the solubility range. About 5x the quantity of the material determined in the preliminary test is weighed into each of three vessels with Teflon lined caps (e.g., centrifuge tubes, flasks). The chosen volume of water is added to each vessel, and the vessels are tightly sealed. The closed vessels are then agitated at 30 °C (A shaking or stirring device capable of operating at constant temperature should be used). After 1 day, one of the vessels is removed and re-equilibrated for 24 hours at the test temperature (20 °C) with occasional agitation. The contents of the vessel are then centrifuged at the test temperature, and the concentration of compounds in the visually clear aqueous phase will be determined by measurement of the phosphorus content using an Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES). The other two flasks are treated similarly after initial equilibration at 30 °C for 2 and 3 days, respectively. If the concentration results from at least the last two vessels do

not differ by more than 15%, the test is satisfactory. The whole test should be repeated, using longer equilibration times if the results from Vessels 1, 2, and 3 show a tendency to increasing values. The pH of each sample should be measured using indicator strips.

Stability at Test Conditions

The test substance will be analyzed for stability and recovery of the test substance using the following procedure:

Solutions for stability under test conditions at a suitable nominal concentration will be prepared and analyzed immediately by HPLC-UV. Recovery will be determined by HPLC-UV. The remaining solutions will be maintained under the same conditions as the solutions used for the water solubility experiment and then analyzed as described above. Stability at test conditions will be evaluated based on comparison of the peak area response obtained at preparation and at the conclusion of the water solubility experiment. The actual method(s) will be documented in the study records and described in the final report.

Methods to Control Bias

All instrumental data will be collected by qualified computer software (PerkinElmer WinLab 32 for ICP-AES and PerkinElmer TotalChrom version 6.3.1 for HPLC-UV). All calculations will be performed by Microsoft Excel™ and will be peer verified for correctness. Intermediate calculation values presented in the final report will be rounded only for discussion or presentation in tables, but the raw values themselves will be retained until the final calculations are complete.

As applicable, bias will be controlled by ensuring:

- The instrument is qualified for use.
- The instrument is calibrated and maintained per SOP.

Proposed Statistical Method(s)

Appropriate statistical methods for the analysis and evaluation of the experimental data will be used at the discretion of the Study Director. Common statistical methods used to evaluate the precision and accuracy of the measurements may include (as appropriate): mean, coefficient of variation, standard deviation, and confidence interval.

To improve data presentation and interpretation, and facilitate report preparation, the Study Director may apply computer programs for spreadsheets (e.g., Excel), graphics presentations (e.g., Word or PowerPoint), and general standard statistics software.

RECORDS TO BE MAINTAINED

Analysts shall document all experimentation such that an experienced scientist can reconstruct the work. Documentation shall include sample identifications, weighings, dilutions, calculations, etc. Additional documentation shall include instrumentation and equipment utilized during the study, as well as documentation of prepared reagents and solutions.

All study data shall be reviewed or verified and maintained in folders in the study activity file. Research notebook(s) shall be placed in the study activity file at the completion of the study.

Other comments, descriptions, calculations, correspondence, etc., shall be placed in the study activity file.

Upon conclusion of the study copies of representative raw data (as appropriate), shall be submitted to the Sponsor. An accurate study file, including original raw data, shall be submitted to the Ricerca Biosciences, LLC Archives, 7528 Auburn Road, Concord, Ohio.

REPORT

A final report will be prepared at the conclusion of the study. The report shall include, but not necessarily be limited to, the following:

- Name and address of the facility performing the study and the dates on which the study was initiated and completed, terminated, or discontinued
- Reference(s) to, and/or a detailed description of, all methods used
- The individual analytical determinations and the average where more than one value was determined for each flask.
- The average of the value for the different flasks which were in agreement.
- The test temperature
- The analytical method employed
- Identification of the test and/or reference substances used in the study
- All deviations and changes from the protocol
- A description of all circumstances that may have affected the quality or integrity of the data
- Name and signature of the Study Director, the names of other scientists or professionals, and the names of supervisory personnel involved in the study
- Statistical methods employed for analyzing the data. A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis.
- Locations where raw data and the final report are to be stored
- The signed and dated statement by the Ricerca Quality Assurance Unit specifying the dates of study inspections and dates the findings were reported to the Study Director and Management, when applicable
- The signed and dated statement by the Study Director describing compliance with the Good Laboratory Practice Standards as specified in 40 CFR 160.

AMENDMENTS AND DEVIATIONS TO THE PROTOCOL

All agreed upon amendments will be expressed in writing, signed and dated by the Study Director. Protocol amendments will also be signed by the Sponsor, or the Sponsor may provide their approval by means of a Sponsor Approval Form. Copies of the signed amendments and any Sponsor Approval Forms will be returned to the Study Director and appended to the protocol.

Deviations from the protocol, if any, will be initiated by the Study Director, documented in the study file, and listed in the final report.

PROTOCOL ACCEPTANCE

Study Title: Determination of Water Solubility of K32

Document Number: 035239-0

Testing Facility: Ricerca Biosciences, LLC
7528 Auburn Road
Concord, OH 44077

Ling-Jen Ferguson — Sep 30, 2016

Ling-Jen Ferguson, Ph.D., Study Director — **Date**
Ricerca Biosciences, LLC

Robert McClanahan — Sept 30, 2016

Robert McClanahan, Ph.D., Management — **Date**
Ricerca Biosciences, LLC

SPONSOR: KOCH AGRONOMIC SERVICES, LLC
SPONSOR APPROVAL DATE: SEPTEMBER 29, 2016

APPENDIX B

K32 Certificate of Analysis

CERTIFICATE OF ANALYSIS

K32

Batch/Lot No: 55700-30-13

Ricerca Sample Code: CS 22050

Test	Test Method	Test Result
Active Ingredient Content and Identification	**HPLC-UV TAN and LC-MS**	**See Table Below**
Phosphorus Content	**ICP**	**12.4%**
Identification and Relative Quantification	**31P-NMR**	**See Table Below**
N-(n-butyl) thiophosphoric triamide (NBPT) Content	**HPLC**	**20.04%**
Conditions:	Refrigerate for prolonged storage (2-8 °C); may be handled at room temperature	
Study Initiation Date:	November 1, 2016	
Study Completion Date:	November 30, 2016	

Active ingredient content by identification of peak using LC-UV TAN and LC-MS

Component	Observed Retention Time (minutes)	Observed %Area	Expected Molecular Mass	Observed molecular mass
1	6.58	0.2	478 and 406 $[M+Na]^+$	406.1 $[M+Na]^+$
2	6.98	1.9	334 $[M+Na]^+$	334.1 $[M+Na]^+$
3	7.48	4.2	262 $[M+Na]^+$	262.1 $[M+Na]^+$
3b	7.76	0.4		334.1 $[M+Na]^+$
4	10.83	0.5	406 $[M+Na]^+$	406.1 $[M+Na]^+$
5	11.06	3.1	334 $[M+Na]^+$	334.1 $[M+Na]^+$
6	11.48	17.5	262 $[M+Na]^+$	262.1 $[M+Na]^+$
7	12.16	30.2	190 $[M+Na]^+$	168.1 $[M+H]^+$
8	12.62	0.2	406 $[M+Na]^+$	406.1 $[M+Na]^+$
9	12.89	0.4	334 $[M+Na]^+$	334.1 $[M+Na]^+$
10	13.55	0.1	252 $[M+Na]^+$	274.1 $[M+Na]^+$
11	17.4 to 30.0	41.1	Not Defined, mixture	Not Defined, mixture

Identification and Relative Quantification using ^{31}P-NMR

3.53 wt% K32	Chemical shifts (ppm)	NMR integral	Concentration (mM/g)*	Relative Ratio (to NBPT)
K32-a	[-0.22 - -0.61]	0.12	0.01	0.01
K32-b	[53.37 - 52.91]	0.33	0.03	0.02
K32-c	[55.73 - 55.43]	0.11	0.01	0.01
K32-d	[58.15 - 57.85]	0.15	0.01	0.01
NBPT	[59.80 - 59.49]	14.42	1.10	1.00
K32-e	[60.07 - 59.80]	7.83	0.60	0.54
K32-f	[60.31 - 60.07]	9.55	0.73	0.66
K32-g	[61.65 - 60.54]	5.48	0.42	0.38
L32-h	[64.97 - 63.38]	8.97	0.69	0.62
Total	[0.00 - 70.00]	46.96	3.60	

**mMol of each compound in per gram of K32*

Ricerca Biosciences, LLC • 7528 Auburn Rd. • Concord, OH 44077
Tel. 440.357.3300 • Fax 440.354.6276 • info@ricerca.com

CERTIFICATE OF ANALYSIS

Penny Miner

Penny Miner, Study Director
AgChem Product Development

November 30, 2016
Date

Phillip Cassidy

Phillip Cassidy, Management
AgChem Product Development

November 30, 2016
Date

1. The objective of this study was to determine the active ingredient content, phosphorus content and mass spectra of K32, to be used as a test, reference or control substance in a study.
2. This study was conducted in accordance with the Good Laboratory Practice Standard, 40 CFR Part 160.135 (b).
3. No deviations occurred from GLP regulations, the protocol, and relevant SOPs.
4. Data for this Certificate of Analysis is archived at the address below under Project Number 035469.
5. Only descriptive statistics were used.

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